The Practicality of an ALS Bend Magnet as the X-Ray Source for solving Structural Biology problems.

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INTRODUCTION

Significant progress in structural biology is occurring at present with the maturation of the technique of protein crystallography carried out at synchrotron radiation sources worldwide. Access to bright, tunable X-ray beamlines limits the pace of progress in structural biology. We have a program to establish the practicality of carrying out structural biology studies on a regular bending magnet at the local ALS synchrotron. Building such a beamline on a low power, high brightness ALS bending magnet source is viewed as being a very cost-effective solution to improving rapid access to local protein crystallography facilities. To this end we have carried out a Multiple Anomalous Dispersion (MAD) experiment at the Co edge and also studied the diffraction data set from weakly diffracting crystals.

EXPERIMENTAL

We carried out experiments on Beamline 7.3.3 to demonstrate the feasibility of data collection and structure determination by multiwavelength anomalous diffraction (MAD) analysis using an ALS bend source. The optical configuration included a channel-cut, germanium monochromator operating with a band pass of ~1/1000. Optimized optics in the new beamline are expected to increase brightness by over fifteenfold over the prototype configuration used on 7.3.3. Several crystalline samples were analyzed, including a designed, α-helical coiled coil and a eukaryotic, transcriptional coactivator/activator complex.

RH3, a designed, trimeric α-helical coiled coil with an engineered metal binding site (Harbury et al., 1998) was used to demonstrate that the bend magnet source and optics can meet the rigorous demands of MAD analysis. The trigonal crystals have unit cell dimensions of 25 x 25 x 141 Å, with a single 12 kDa trimer in the asymmetric unit. The metal binding site, consisting of paired γ-carboxyglutamate residues, offers the flexibility to incorporate different metal ions for phasing. Crystals grown in the presence of Co^{2+}, which has four anomalous electrons, were analyzed. Complete data sets were collected to 1.9 Å resolution at eight energies across the cobalt edge (~7709 eV) and processed in an automated mode with a SMARTflm routine developed at UCB (James Holton and T.A., unpublished). Data collection was completed in ~10 hours, and processing yielded merging R values of 5-9% and overall I/σI values of 11-20. The data were of average quality, reflecting the rapid collection speed (Table 1).

Data collected at the energies of the maximum anomalous and dispersive signals (Fig. 1) were used in concert with the low energy reference data to identify the Co^{2+} binding sites with the program SHELX (Sheldrick, 1990). Initial phases were calculated using MLPHARE (CCP4, 1994), resulting in excellent phasing statistics including phasing powers above 2.0 (Table 1). These phases were subsequently improved by solvent flattening with DM (CCP4, 1994), yielding electron density maps that were extremely clear and readily interpretable (Fig. 2). Automated model building and refinement with wARP (van Asselt et al., 1998) produced initial R and free R-values of 0.239 and 0.320, respectively, prior to any manual adjustment of the model. The entire process, from data collection to preliminary refinement of the model, was completed in 36 hours.

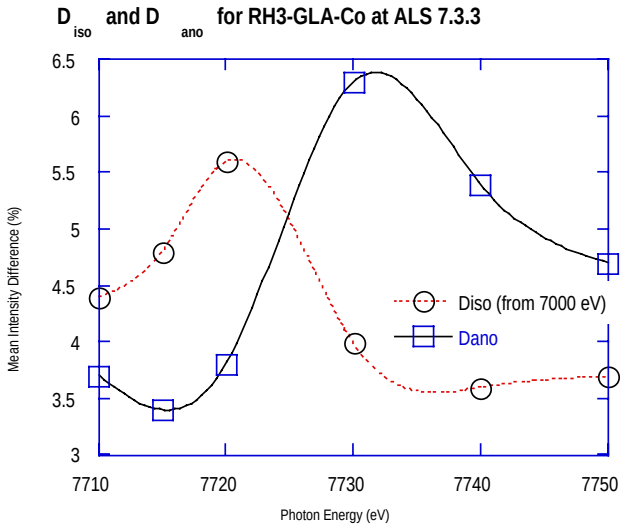

Figure 1. Dispersive (D_{iso}) and anomalous differences as a function of data collection energy for RH3. The dispersive difference was calculated versus the low energy reference energy of 7700 eV. The dispersive difference was maximized at 7720 eV and the anomalous difference was maximized at 7730 eV. Significantly, the maximum dispersive difference did not occur at the apparent midpoint of the anomalous signal.

ALS Beamline 7.3.3, 12/98, 50-1.9 Å resolution, Co^{2+} complex							
Energy	**R_{sym}**	**R_{anom}**	**I/σI**	**Complete-Ness (%)**	**Multi-plicity**	**Phasing power ano/disp**	**Cullis R ano/disp**
7000	0.058	0.043	14.2	80.1	3.1	0.14/2.38	0.99/0.54
7710	0.062	0.048	11.6	93.2	3.1	0.17/1.14	0.98/0.79
7715	0.051	0.043	13.3	93.6	3.3	0.26/0.82	0.97/0.88
7720	0.079	0.050	18.0	84.7	7.2	1.02/ -	0.68/-
7730	0.068	0.086	12.8	94.4	4.9	1.36/2.05	0.59/0.59
7740	0.052	0.077	12.6	93.8	3.3	1.21/2.26	0.63/0.56
7750	0.055	0.061	12.7	94.2	3.3	0.82/2.17	0.75/0.57
8000	0.094	0.102	20.1	92.8	3.2	0.46/1.82	0.89/0.66
Mean figure of merit (25-1.9 Å): 0.776							
Crystallographic R/R_{free} (25-1.9 Å): 0.239/0.320 (824 atoms built and refined with wARP 5.0)							
SSRL Beamline 1-5, 50-2.0 Å resolution, Ni^{2+} complex							
8342.0	0.084	0.036	20.8	86.6	8.1	1.01/4.37	0.70/0.35
8348.2	0.081	0.076	21.4	86.6	8.2	1.69/2.22	0.51/0.58
8900.0	0.084	0.049	16.4	84.5	7.0	1.30/-	0.60/0.35
Mean figure of merit (25-1.9 Å): 0.831; after solvent flattening: 0.899.							
Crystallographic R/R_{free} (25-1.9 Å): 23.3/26.9 (Δbonds = 0.007 Å, Δangles = 1.07°; refined with CNS (Brunger, 1998)							

Table 1. RH3 data collection and phasing statistics. Space group $P3_1$, a=b=25, c=141 Å.

Figure 2. MAD-phased, experimental electron density map of RH3 (25-1.9 Å resolution) contoured at 1σ clearly shows helical trimer and the core isoleucines packing in a knobs-into-holes arrangement. View is along the pseudo-three-fold axis of the trimer showing a cross section through the parallel helices.

As a comparison, data were collected from a comparable, isomorphous crystal of RH3 complexed with Ni^{2+} at Beamline 1-5 at SSRL. Data collection at four energies required four full days of beam time. The quality of the data collected on ALS Beamline 7.3.3 compares favorably with the data collected at SSRL (Table 1), and clearly the ALS bend source offers superior brightness and speed.

To explore the feasibility of using ALS Beamline 7.3.3 for data collection on a more weakly diffracting experimental system, we analyzed crystals of a complex between the transcriptional coactivator DCoH and the dimerization domain of its cognate activator, HNF-1. The crystals have the symmetry of space group $P2_1$, with 62 kDa in the asymmetric unit. Data collected at a single energy required ~10 hours of exposure, compared to four days on a laboratory source or three hours at Beamline 7-1 at SSRL. The resolution and accuracy of the data were better than data from a laboratory source (not shown), but slightly worse than the data collected on the same crystal at SSRL Beamline 7-1 (Table 2). Although the reduced data quality directly reflects the comparably lower flux of Beamline 7.3.3, it is important point out that SSRL 7.1 is a wiggler beamline, while 7.3.3 is an ALS bend magnet.

	ALS Beamline 7.3.3, 12/98		SSRL Beamline 7-1	
Resolution (Å)	**R_{merge}**	**I/σI**	**R_{merge}**	**I/σI**
50 - 7.39	0.030	37.9	0.020	46.7
5.87	0.027	34.1	0.023	59.8
5.13	0.029	30.6	0.022	63.6
4.66	0.028	32.8	0.021	66.8
4.33	0.030	32.4	0.023	64.6
4.07	0.032	28.6	0.025	64.1
3.87	0.034	27.4	0.027	63.3
3.70	0.040	24.5	0.030	59.2
3.56	0.046	21.6	0.035	54.7
3.43	0.054	18.9	0.038	50.9
3.33	0.060	16.5	0.041	47.6
3.23	0.088	11.9	0.050	39.9
3.15	0.088	10.1	0.055	36.1
3.07	0.106	8.7	0.066	31.6
3.00	0.129	7.2	0.075	27.4
Overall	**0.041**	**24.0**	**0.030**	**54.4**

Table 2. DCoH/HNF-p1 data collection statistics. Space group: $P2_1$, a=49.4, b=83.0, c=70.4 Å, β= 97.4°.

These results demonstrate the feasibility of solving structures rapidly using an ALS bend magnet source. In particular, both energy resolution and intensity are sufficient for MAD analysis and for weakly diffracting crystals. Proposed enhancements to the optical system over the current 7.3.3 configuration would be expected to provide over an order of magnitude increase in brightness and a commensurate increase in data quality and throughput. Taken together, this data clearly shows that an inexpensive ALS bend magnet can readily compete with far more costly wiggler facilities.

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